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The Effect of Controlled Ozone Exposure on Human Lumphocyte Function

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16. ABSTRACT

The effects of ozone (O_3) on cell-mediated immunity were studied in 16 human subjects exposed to 1176 $\mu g/m^3$ O_3 (0.6 ppm) for 2 hr in an environmentally controlled exposure chamber. Venous blood samples were taken before and immediately after controlled air and O_3 exposures, as well as at 72 hr, 2 and 4 weeks, and at one random time at least 1 month after treatment. The relative frequency of T lymphocytes in blood and the in vitro blastogenic response of lymphocytes to phytohemagglutinin (PHA), concanavalin A (Con A), pokeweed mitogen (PWM), and Candida albicans were determined. During the course of the experiment, no statistically significant changes were observed in the number of T lymphocytes that form spontaneous rosettes with sheep erythrocytes. The response of T lymphocytes to PHA was significantly reduced ($P < 0.5$) in samples taken at 2 and 4 weeks following O_3 exposure. Normal response PHA observed at 2 months post-O_3 exposure. No statistically significant changes in lymphocyte responses to Con A, PWM, or Candida were seen. These results show that one 2 hr exposure of humans to 0.6 ppm O_3 may lead to a transient suppression of the PHA-stimulated blastogenic transformation of peripheral blood lymphocytes. The data indicate that the blastogenic response to PHA of human lymphocytes is exquisitely sensitive to O exposure and could serve as a bioassay for evaluating subtle changes in cellular immunity induced by O_3 and possibly other pollutants.

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The Effect of Controlled Ozone Exposure on Human Lymphocyte Function[1]

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The effects of ozone (O_3) on cell-mediated immunity were studied in 16 human subjects exposed to 1176 $\mu g/m^3$ O_3 (0.6 ppm) for 2 hr in an environmentally controlled exposure chamber. Venous blood samples were taken before and immediately after controlled air and O_3 exposures, as well as at 72 hr, 2 and 4 weeks, and at one random time at least 1 month after treatment. The relative frequency of T lymphocytes in blood and the *in vitro* blastogenic response of lymphocytes to phytohemagglutinin (PHA), concanavalin A (Con A), pokeweed mitogen (PWM), and *Candida albicans* were determined. During the course of the experiment, no statistically significant changes were observed in the number of T lymphocytes that form spontaneous rosettes with sheep erythrocytes. The response of T lymphocytes to PHA was significantly reduced ($P < 0.05$) in samples taken at 2 and 4 weeks following O_3 exposure. Normal response to PHA was observed at 2 months post-O_3 exposure. No statistically significant changes in lymphocyte responses to Con A, PWM, or *Candida* were seen. These results show that one 2 hr exposure of humans to 0.6 ppm O_3 may lead to a transient suppression of the PHA-stimulated blastogenic transformation of peripheral blood lymphocytes. The data indicate that the blastogenic response to PHA of human lymphocytes is exquisitely sensitive to O_3 exposure and could serve as a bioassay for evaluating subtle changes in cellular immunity induced by O_3 and possibly other pollutants.

INTRODUCTION

One of the most thoroughly studied inhaled toxicants is the oxidant gas ozone. Ozone (O_3) is found in significant amounts in urban and industrial areas and may account for as much as 90 percent of the total oxidant (National Air Pollution Control Administration, 1970). Extensive studies in animals have shown that O_3 is highly cytotoxic at high concentrations (Stokinger, 1965) and produces stimulation of lung cell metabolism at lower (ambient) concentrations (Chow and Tappel, 1973). A recent study has shown (Peterson *et al.*, 1978) that a single 4-hr exposure of humans to 0.4 ppm O_3 may temporarily suppress the *in vitro* blastogenic response of T lymphocytes stimulated by phytohemagglutinin (PHA), suggesting that a suppression in the proliferative capacity of circulating lymphocytes may be an important immunologic indicator of O_3 toxicity.

[1] The protocol was approved by the Committee for Protection of Human Subjects of the University of North Carolina, School of Medicine, Chapel Hill, NC. These data have been presented in part at the 4th FDA Science Symposium, "Inadvertent Modification of the Immune Response," August 28–30, 1978, Annapolis, MD.

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To approximate the actual exposure experience observed in high-oxidant pollution areas (Monitoring and Air Quality Trends Report, 1973) and its effect on the cellular immune mechanisms in humans, a study was designed to characterize membrane properties and the blastogenic *in vitro* response of lymphocytes from humans exposed for 2 hr to 0.6 ppm (1176 $\mu g/m^3$) O_3.

MATERIALS AND METHODS

Subject Selection

Sixteen healthy, nonsmoking, male volunteers between the ages of 19 and 27 participated in this experiment. Subjects were selected by screening with a Minnesota Multiphasic Personality Inventory (MMPI), a complete medical history, a physical examination, and a complete blood count. Suitable subjects were candidates who were within normal limits on all screening tests. After obtaining informed consent to participate voluntarily in the study, final selection of the subjects was determined by an adequate performance in a chamber training session. None of the subjects had a history of allergic, respiratory, or cardiac illness or had used any medications for at least one week prior to testing.

Exposure Studies

Exposures to O_3 or clean air took place in a controlled environmental chamber (2.4 × 2.4 × 2.4 m) constructed of 1.3-cm Plexiglas (Strong *et al.*, 1977). O_3 was generated from pure oxygen using the OREC O_3 generator and was injected into the chamber air supply duct at a low pressure point. The air–O_3 mixture was dispersed into the chamber through a perforated grill in the chamber ceiling. O_3 concentration was continuously monitored using a chemiluminescent analyzer (Bendix, Ronceverte, W.Va.) and maintained to within ± 5 percent of the desired concentration. Chamber temperature was maintained at 21–24°C and relative humidity at 40–60%.

Subjects were seated and resting in the chamber for the 2-hr exposure period except for two 15-min exercise periods on a bicycle ergometer (Quinton Model 844, Quinton Instrument, Seattle, Wash.) beginning at 30 and 90 min into the exposure regimen. The exercise was sufficient to approximately double resting heart rate as monitored by electrocardiograph (EKG). Each subject served as his own control (Campbell and Stanley, 1966) by breathing filtered ambient laboratory air on control day (Friday a.m.) followed by 0.6 ppm O_3 on exposure day (Monday a.m.) in an otherwise identical exposure protocol. It is noted that this exposure design also incorporated pulmonary function measurements and has been used earlier by Savino *et al.* (1978).

Venous blood samples, used for immunologic measurements for both the air and 0.6 ppm O_3 exposures, were taken immediately prior to exposure (baseline); immediately after each exposure; on days 3, 14, and 28; and again at one random time at least 1 month after O_3 exposure.

Lymphocyte Blastogenic Transformation

Peripheral blood was obtained from fasting individuals in sterile, siliconized glass tubes each containing 15 units/ml preservative-free heparin. Purified lymphocyte suspensions were prepared by Ficoll–Hypaque (Litton Bionetics, Inc.,

Kensington, Md.) gradient sedimentation (Oppenheim and Schecter, 1976). After centrifugation at 400 × *g* for 40 min at 12°C, the cells on the gradient interface were collected and washed three times with RPMI 1640 medium (Grand Island Biological Co., Grand Island, N.Y.) containing 5 percent heat inactivated fetal calf serum. The cell pellet was suspended in RPMI 1640 medium supplemented with 50 units penicillin G, 50 μg streptomycin, and 2 mM L-glutamine per 1 ml, and 20% heat-inactivated fetal calf serum. Cell viability was greater than 98% as measured by trypan blue dye exclusion. Lymphocytes were added to wells in microtiter plates (Falcon Plastics, Div. Becton, Dickinson Co., Oxnard, Calif.) in 100-μl volumes containing 10^5 cells for unstimulated and mitogen-stimulated cultures and 2.5×10^5 cells for antigen-stimulated cultures. Dose–response curves for mitogens were determined in twofold titration experiments with lymphocytes from normal donors. Optimal doses of mitogens were as follows: PHA (purified PHA, Burroughs Wellcome Co., Research Triangle Park, N.C.) 0.5 to 32 μg/ml with a maximal stimulation at 2 μg/ml, concanavalin A (Con A, Sigma Chemical Co., St. Louis, Mo.) 3.2 to 50 μg/ml with a maximal response at 12.5 μg/ml, and pokeweed mitogen (PWM, Grand Island Biological Co., Grand Island, N.Y.) 0.16 to 10 μl/ml with a maximal response at 2.5 μl/ml. Optimal stimulation of lymphocytes with *Candida albicans* extract (Hollister–Stier Laboratories, Atlanta, Ga.) occurred with concentrations of from 5 to 20 μl/ml. Three to five different concentrations of each mitogen and the antigen *Candida albicans* were used to evaluate lymphocyte responses of exposed individuals. All determinations were performed in triplicate, and included control cultures without stimulants. Cultures were incubated at 37°C in 5% CO_2 in a humidified atmosphere for 4 days; cells were pulsed for the last 22 hr with [2-^{14}C]thymidine, 0.05 μCi/well, specific activity 50 mCi/mmole (New England Nuclear, Boston, Mass.). Lymphocytes were harvested using a MASH automatic cell harvester (Microbiological Associates, Bethesda, Md.) onto glass fiber filter paper, and DNA was precipitated with ice-cold 5% trichloroacetic acid. Filters were dried and placed in vials to which 10 ml of Liquifluor (NEN, Boston, Mass.) scintillation fluid was added. Vials were counted in a liquid scintillation counter (Packard Instrument Co., Downers Grove, Ill.). Raw data in counts per minute (cpm) were transformed logarithmically for statistical analysis. Computations of data from preair and up to 4 weeks post-O_3 exposure were made by analysis of variance with repeated measurements (Winer, 1963). Three hypotheses were tested. The first hypothesis was that there was no difference in the response with air (preair minus postair). The second hypothesis was that there was no difference in the response with O_3 (pre-O_3 minus post-O_3). These first two hypotheses were tested by individual degree-of-freedom comparisons. The third hypothesis was that the post-O_3, 72-hr post-O_3, and 2- and 4-weeks post-O_3 means were no different from the pre-O_3 mean. This hypothesis was tested by using Dunnett's test for comparing all means to a control mean (Dunnett, 1955).

A paired *t* test on the $\log_{10}$ (cpm) was used to test for differences from preair in individuals tested 2 to 6 months post-O_3 exposure ($P > 0.05$).

E-Rosettes

The number of spontaneous rosettes formed between human lymphocytes and sheep erythrocytes was determined as described by Mendes *et al.* (1973) and Lay

et al. (1971). Briefly, lymphocyte suspensions were prepared as for the transformation studies, except that the cells were washed with Hanks' balanced salt solution (HBSS, Grand Island Biological Co., Grand Island, N.Y.). To detect spontaneous sheep erythrocyte rosette formation, lymphocyte suspensions containing 2×10^6 cells/ml were mixed with an equal volume of a 0.5% suspension of sheep erythrocyte (E) in HBSS. This suspension was then centrifuged at $200 \times g$ for 5 min, held in an ice-water bath for 1 hr, then gently resuspended, and rosettes were counted microscopically in a hemacytometer. At least 200 lymphocytes were counted from each of two replicate tubes and only rosettes possessing three or more adhering erythrocytes were counted. The results were reported as the mean percentage of rosetting lymphocytes. Data were analyzed using a single factor analysis of variance with repeated measures with the single factor being time (preair, postair, pre-O_3, post-O_3, 72-hr post-O_3, and 2-weeks post-O_3).

RESULTS

Lymphocyte Counts

The total number of white blood cells (WBC) and the percentages of lymphocytes, neutrophils, and monocytes per mm^3 of blood before air and O_3 exposures were within normal limits (Table 1). A significant ($P < 0.05$) increase in WBCs per mm^3 was seen 2 hr postexposure to either air or O_3 but no significant changes were noted at other times tested. Following the 2-hr air or O_3 exposure, the percentages of lymphocytes per mm^3 was decreased, and the percentage of polymorphonuclear (PMN) cells increased. These differences were, however, not statistically different. No change was noted in the percentage of monocytes.

Lymphocyte Blastogenic Transformation

The blastogenic responses of lymphocytes from 16 subjects for pre- and imme-

TABLE 1
BLOOD LEUKOCYTES PRE- AND POST-AIR AND OZONE EXPOSURES

Time	Total WBC (number/mm^3)	Lymphocytes, (%)	PMNs, (%)	Monocytes, (%)
Preair	5,520 ± 280	42.1	48.8	4.3
2 hr postair	6,270* ± 270	34.3	57.3	4.7
Preozone	5,350 ± 300	40.4	49.4	5.2
2 hr postozone	6,500* ± 450	30.8	62.0	3.5
72 hr postozone	5,280 ± 360	41.1	50.8	4.6
2 weeks postozone	5,500 ± 180	40.0	51.9	5.0
4 weeks postozone	5,880 ± 250	37.4	55.3	3.7

* $p < 0.05$.

diately postair and O_3 exposures are presented in Figures 1–3. The subjects' mean responses ± standard error (SE) to 2 μg/ml PHA showed a slight decrease after air and O_3 exposures; however, these were not statistically different from 0-hr values (Fig. 1). An increased responsiveness to concentrations of Con A was seen immediately after air and O_3 exposures (Fig. 2), but the group means did not differ statistically from those for preexposure. Responses to PWM and *Candida albicans* were increased at all concentrations after the 2-hr exposure to air and slightly decreased at optimal response concentrations after the 2-hr O_3 exposure (Fig. 3); however, these changes were not statistically significant.

A follow-up of optimal blastogenic responses following O_3 exposure is shown in Fig. 4. Significant ($P < 0.05$) decreases in the mean PHA responsiveness were found in samples collected at 2 and 4 weeks following O_3 exposure (decrease from 14,771 ± 1742 cpm at 0 hr to 10,248 ± 1363 cpm and 8,701 ± 928 cpm at 2 and 4 weeks, respectively). The fluctuation of PHA responses in individual subjects with time was further investigated by using the preair, postair, and pre-O_3 measurements to calculate a pooled-within-subjects estimate of the standard deviation (SD) of DNA counts. A "responder" was defined as a subject whose DNA count at some subsequent time decreased by more than two times the SD. Using this value and comparing the change from pre-O_3, there were two responders at 72 hr,

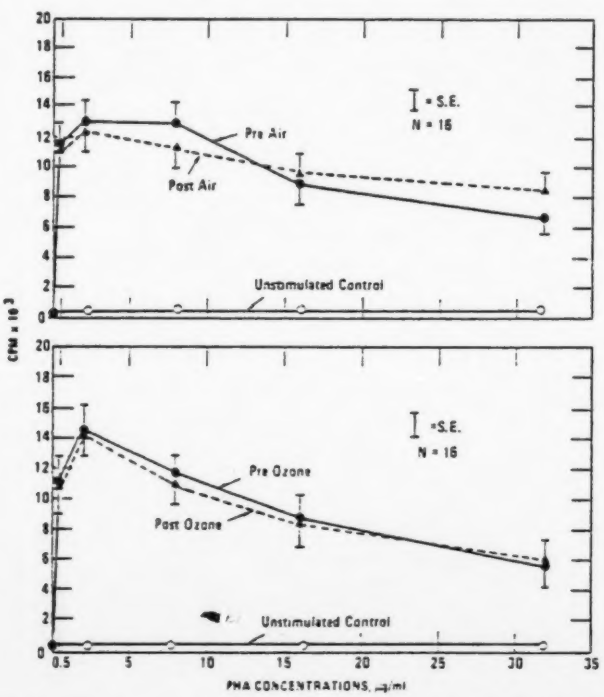


FIG. 1. Dose response of [2-^{14}C]thymidine incorporation (cpm) in lymphocyte cultures stimulated with different concentrations of PHA for 16 individuals before and immediately after exposure to air and O_3.

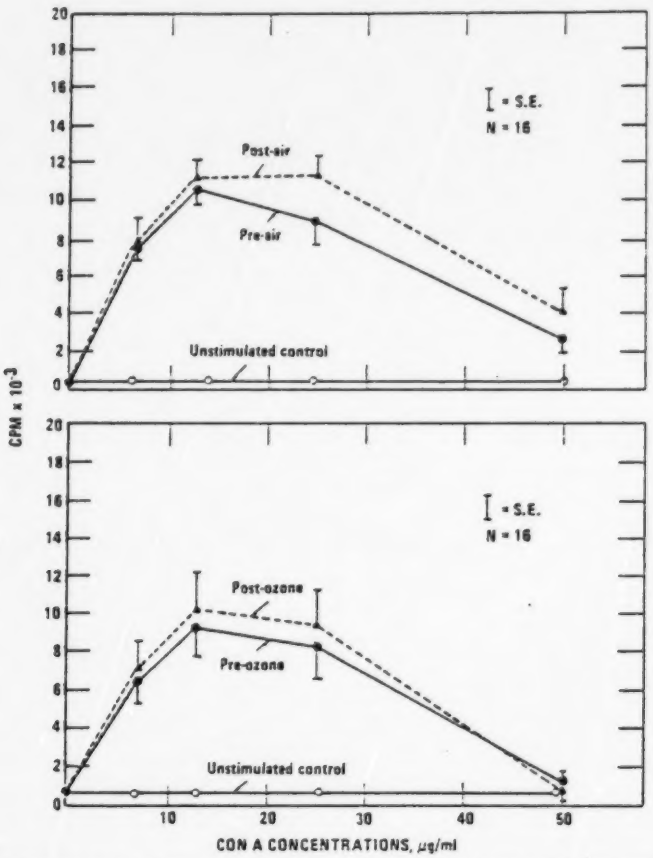


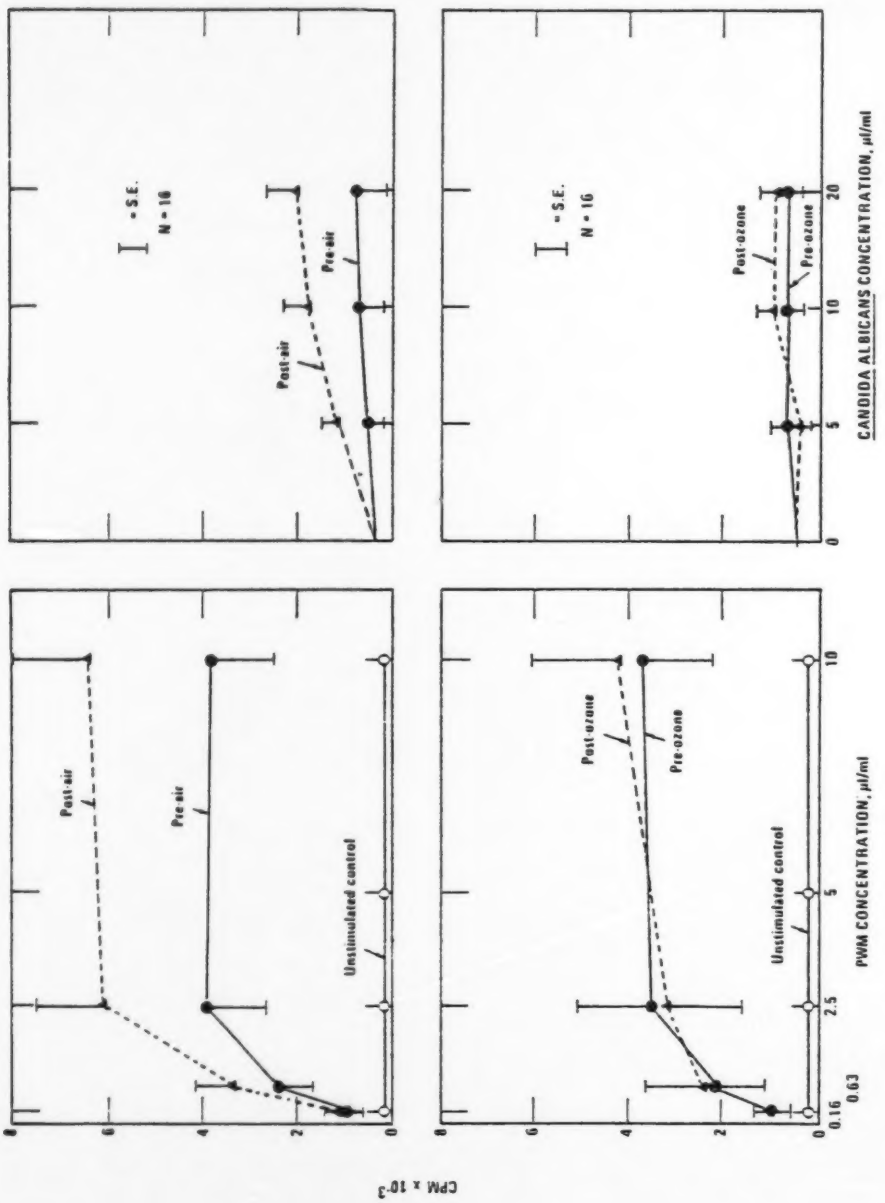
FIG. 2. Dose response of [2-^{14}C]thymidine incorporation (cpm) in lymphocyte cultures stimulated with different concentrations of Con A for 16 individuals before and immediately after exposures to air and O_3.

three responders at 2 weeks, and five responders at 4 weeks following exposure to O_3.

The group means for Con A and PWM showed an increase in responsiveness at 72 hr post-O_3 exposure; however, this increase was not statistically different from that at 0 hr. Mean values obtained for Con A, PWM, and *Candida albicans* at 2 and 4 weeks post-O_3 exposure were not significantly different from preexposure values. Background responses of unstimulated cultures were low at all times, less than 200 cpm.

The responsiveness of lymphocytes to PHA was again retested at three different

FIG. 3. Dose response of [2-^{14}C]thymidine incorporation (cpm) in lymphocyte cultures stimulated with different concentrations of PWM and *Candida albicans* for 16 individuals before and immediately after exposures to air and O_3.


± S.E.
N = 16
Post-air
Pre-air
Unstimulated control
Post-ozone
Pre-ozone
PWM CONCENTRATION, µl/ml
CANDIDA ALBICANS CONCENTRATION, µl/ml
CPM x 10^-3

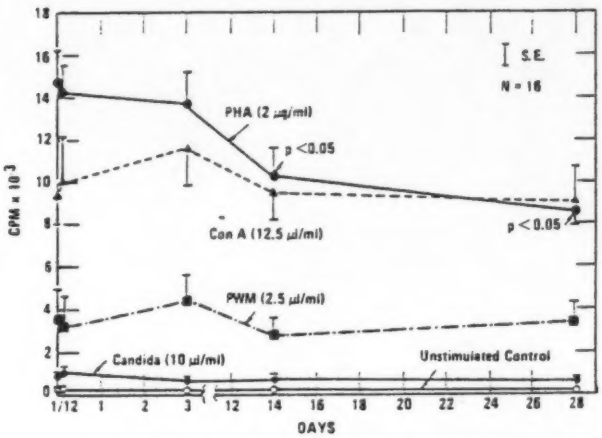


FIG. 4. Blastogenic responses of lymphocytes (cpm) in humans following exposure to O_3. Each point represents the mean of lymphocyte cultures from 16 individuals, each done in triplicate.

times (Fig. 5). Four of the sixteen subjects studied returned at 2 months, two at 4 months, and three between 5 and 6 months post-O_3 exposure. Lymphocytes of the nine subjects analyzed exhibited PHA responses statistically not different from those obtained at 0 hr.

E-Rosettes

The percentages of T lymphocytes forming spontaneous rosettes with sheep erythrocytes for pre- and post- air and O_3 exposures are given in Table 2. Each value represents the mean ± SE of 20 observations for each time period. The analysis of variance showed a significant difference among subjects in the percentage of T-cell rosettes; however, there were no significant differences in the means.

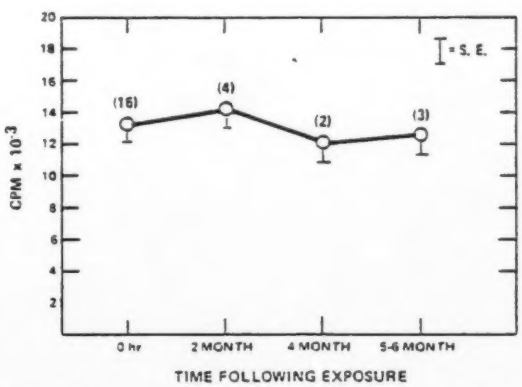


FIG. 5. Follow-up of mean PHA (2 μg ml) responses (cpm) in nine (9) subjects after exposure to O_3; number in parentheses represents the number of individual subjects tested.

TABLE 2
T-ROSETTES PRE- AND POST-AIR AND OZONE EXPOSURES

Time	T-Rosettes (%)	
	Mean	SE
Preair	37.7	1.7
2 hr postair	35.7	2.4
Preozone	39.1	2.2
2 hr postozone	39.3	1.9
72 hr postozone	37.1	1.9
2 weeks postozone	37.8	2.1

COMMENT

Our studies indicate that one 2-hr exposure to 1176 $\mu g/m^3$ (0.6 ppm) O_3 may give rise to a transient suppression in the PHA response of peripheral blood T lymphocytes from healthy human subjects. Suppression of the PHA-stimulated response occurred at 2 and 4 weeks post-O_3 exposure. Responses to PHA, measured in 56% of the subjects studied, returned to normal in 2 months. It is important to note that the current proposed U.S. Ambient Air Quality Standard for photochemical oxidants is 196 $\mu g/m^3$ (0.12 ppm): 0.6 ppm used in these studies has been found on high-smog days in certain areas of southern California (Monitoring and Air Quality Trends Report, 1973).

The mechanism of action of O_3 on lymphocytes is at present open to speculation. The previous studies suggest that O_3 is "radiomimetic" and that the mechanism of its action may be, in part at least, through free radicals, a concept hypothesized for ionizing radiation (Brinkman *et al.*, 1964). It is believed that the major pathway for the toxicity of O_3 involves oxidation of membrane-unsaturated fatty acids to produce ozonides, hydroperoxidases, and other long-lived free radicals (Buckley *et al.*, 1976; Goldstein and Balcum, 1967). These free radicals may undergo chain reactions by which additional free radicals are produced, causing further cell damage. It is not known if O_3 is present in blood to initiate free radical information, or if such a chain reaction begins in lung and extends to other tissues. The hyporesponsiveness of lymphocytes to PHA as seen at 2 and 4 weeks post-O_3 exposure may indicate that circulating cells might be altered as they traffic through the pulmonary vasculature or through pulmonary lymphatics and after a variable period of residence in the lymph nodes or spleen, they reenter the circulation directly. It is conceivable that O_3 or its intermediates interfere with the cellular mechanism of lymphocytes by oxidizing sulfhydryl groups found on surfaces of these cells (Mehrishi, 1972).

The relative proportion of T cells forming high-affinity E-rosettes (1 hr in ice bath) not reduced at 2 and 4 weeks post-O_3 exposure, may indicate that O_3 affects predominantly the subpopulation of T cells which do not form high-affinity E-rosettes. These results would also indicate that within the population of T lymphocytes, the subpopulation which forms high affinity E-rosettes is relatively O_3 resistant. Similar findings have been obtained with subjects undergoing radiation therapy (Raben *et al.*, 1976).

The use of a variety of mitogenic agents for *in vitro* blastogenic response of peripheral blood lymphocytes and the use of membrane surface receptors of lymphocytes have contributed to a partial identification of lymphocyte subpopulations transiently impaired by O_3. The *in vitro* blastogenic response of peripheral blood lymphocytes to PHA appears to be exquisitely sensitive to O_3 exposure and could potentially serve as a bioassay for detecting subtle O_3 and other pollutant-induced functional changes in cell-mediated immunity in humans. This information is needed to set pollutant standards for human exposure.

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The protocol was approved by the Committee for Protection of Human Subjects of the University of North Carolina, School of Medicine, Chapel Hill, N.C.

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REFERENCES

Brinkman, R., Lamberts, H. B., and Veninga, T. S. (1964). Radiomimetic toxicity of ozonized air. *Lancet* 1, 133–136.

Buckley, R. D., Hackney, J. D., Clark, K., and Posin, C. (1976). Biochemical responses of humans to gaseous pollutants. Presented at the BEEP Symposium, EPA, Cincinnati.

Campbell, D. T., and Stanley, J. C. (1966). "Experimental and Quasi-experimental Designs for Research." Rand McNally College Publishing, Chicago.

Chow, C. K., and Tappel, A. L. (1973). Activities of pentose shunt and glycolytic enzymes in lungs of ozone-exposed rats. *Arch. Environ. Health* 26, 205–208.

Dunnett, C. W. (1955). A multiple comparison procedure for comparing several treatments with a control. *J. Amer. Statist. Assoc.*, 50, 1096–1121.

Goldstein, E., and Balcum, O. J. (1967). Effects of ozone on lipid peroxidation in the red blood cell. *Proc. Soc. Exp. Biol. Med.* 26, 356–358.

Lay, W., Mendes, N. F., Bianco, C., and Nussenzweig, V. (1971). Binding of sheep red blood cells to a large population of human lymphocytes. *Nature* 230, 531.

Mehrishi, J. N. (1972). Surface molecular components of human lymphocytes. *Int. Arch. Allergy* 42, 69.

Mendes, N. F., Tolnai, M. E., Silveira, N., Gilbertsen, R., and Metzgar, R. S. (1973). Technical aspects of the rosette tests used to detect human complement receptor (B) and sheep erythrocyte-binding (T) lymphocytes. *J. Immunol.* 111, 860.

"Monitoring and Air Quality Trends Report" (1973). Publication No. EPA-450/1-74-007. Research Triangle Park, N.C.

National Air Pollution Control Administration (1970). "Nationwide Inventory of Air Pollution Emissions (1968)." Raleigh, N.C.

Oppenheim, G. G., and Schecter, B. (1976). "Manual of Clinical Immunology" (N. R. Rose and H. Friedmen, Eds.), pp. 81–84.

Peterson, M. L., Rummo, N., House, D., and Harder, S. (1978). The effect of ozone on human immunity: *In vitro* responsiveness of lymphocytes to phytohemagglutinin. *Arch. Environ. Health* 33, 59.

Raben, M., Walach, M., Galili, U., and Schlesinger, M. (1976). The effect of radiation therapy on lymphocyte subpopulations in cancer patients. *Cancer* 37, 1418.

Savino, A., Peterson, M. L., House, D., Turner, A. G., Jeffries, H. E., and Baker, R. (1978). The effect of ozone in human cellular and humoral immunity: Characterization of T and B lymphocytes by rosette formation. *Environ. Res.* 15, 65–69.

Stokinger, H. E. (1965). Oxone toxicology: A review of research and industrial experience. *Arch. Environ. Health* 10, 719–731.

Strong, A. A., Penley, R., and Knelson, J. H. (1977). "Human Exposure System for Controlled Ozone Atmospheres." Publication No. EPA-600/1-77-048. Research Triangle Park, N.C.

Winer, B. J. (1963). "Statistical Principles in Experimental Design." McGraw–Hill, New York.